

THE BREEDING SYSTEM AND GENE FLOW IN EUCALYPTUS URNIGERA

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Introduction

The stepped cline from green to glaucous morphs of E. urnigera with increasing altitude on Mt. Wellington, Tasmania, is one of the classic examples of clines in forest trees (Barber and Jackson 1957; Endler 1977). A study of the breeding system of E. urnigera was undertaken to provide a basis for investigations of gene flow and the dynamics of this cline.

Clinal variation

The cline in seedling glaucousness is less steep than the cline in the adult population (Fig. 1), which suggests that selection against glaucous morphs at low altitude and against green morphs at high altitudes would be necessary if the cline is in equilibrium. There are also clines in vegetative and reproductive morphology and physiological attributes associated with the cline in glaucousness (Barber and Jackson 1957; Barber 1965; Thomas and Barber 1974 a,b -

Fig. 1). Of particular relevance is the cline in flowering time which more or less coincides with the stepped cline in glaucousness. The mean peak flowering time of the low altitude, green populations is over 3 months earlier than the high altitude, glaucous populations (Fig. 1). Preliminary results from open-pollinated families suggests that at least a component of this variation is heritable. While there is some overlap in flowering time at the individual level, this variation in flowering time must constitute a significant barrier to pollen-mediated gene flow along the cline. This barrier is particularly important in view of the significance of stepped clines in speciation theory.

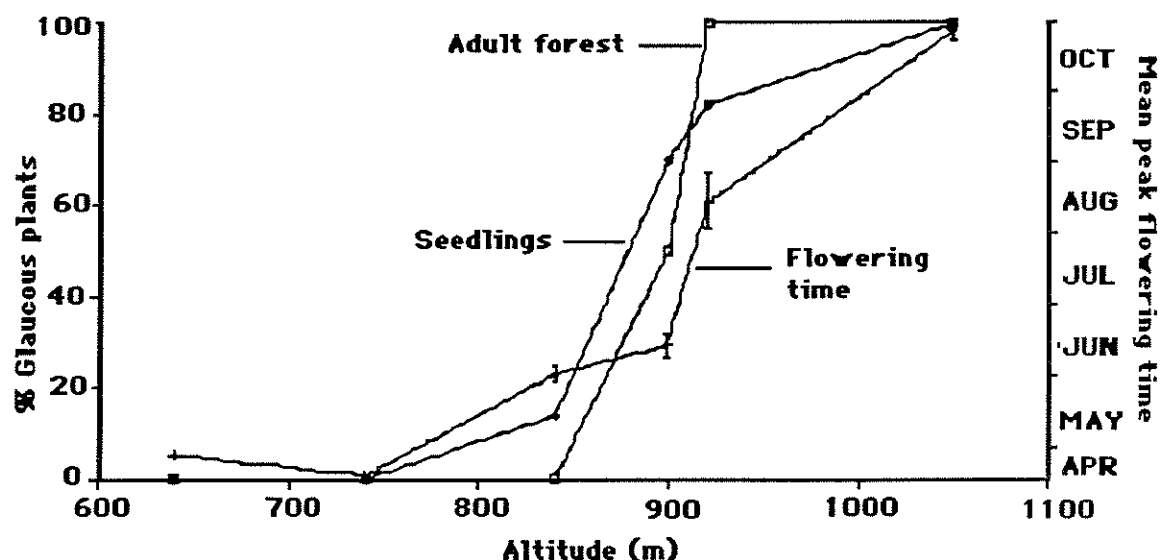


Fig. 1 Clinal variation in adult and seedling glaucousness and mean peak flowering time (1987) of *E. urnigera* with increasing altitude on Mt. Wellington.

Post-anthesis floral development

The time from anthesis (operculum lift - day 0) to senescence of individual flowers spanned approximately 1 month (Fig. 2). Free pollen was evident at anthesis and was abundant until day 10, after which a rapid decline occurred (Fig. 2). Very little free pollen was observed on either the inner or outer whorls of stamens after day 20. Stigma receptivity (as indicated by flattening of the stigma, production of a sticky exudate, a positive esterase test and the commencement of pollen tube growth) commenced about 13 days after anthesis and nearly all stigmata were receptive by day 20 (Fig. 2). Individual flowers are thus clearly protandrous. A small, 15% increase in the relative length of the style occurred following anthesis (c.f. *E. regnans* 67% - Griffin and Hand 1978).

Pollen tubes appear to take approximately 4 days to reach the base of the style. Nectar production peaked immediately prior to stigma receptivity (Fig. 2). However, this peak is broad and nectar is evident just after anthesis and production continued until anther senescence. Treatments preventing pollination resulted in senescence of the anthers being delayed by nearly 2 weeks and early senescence is possibly induced by pollen germination or early tube growth.

The period from anthesis to full stigma receptivity is one of the longest so far recorded in the genus which probably partly results from low temperatures during flowering. The species grows at high altitudes and flowers during winter when snow falls are common. Such winter flowering is rare in subalpine eucalypt species.

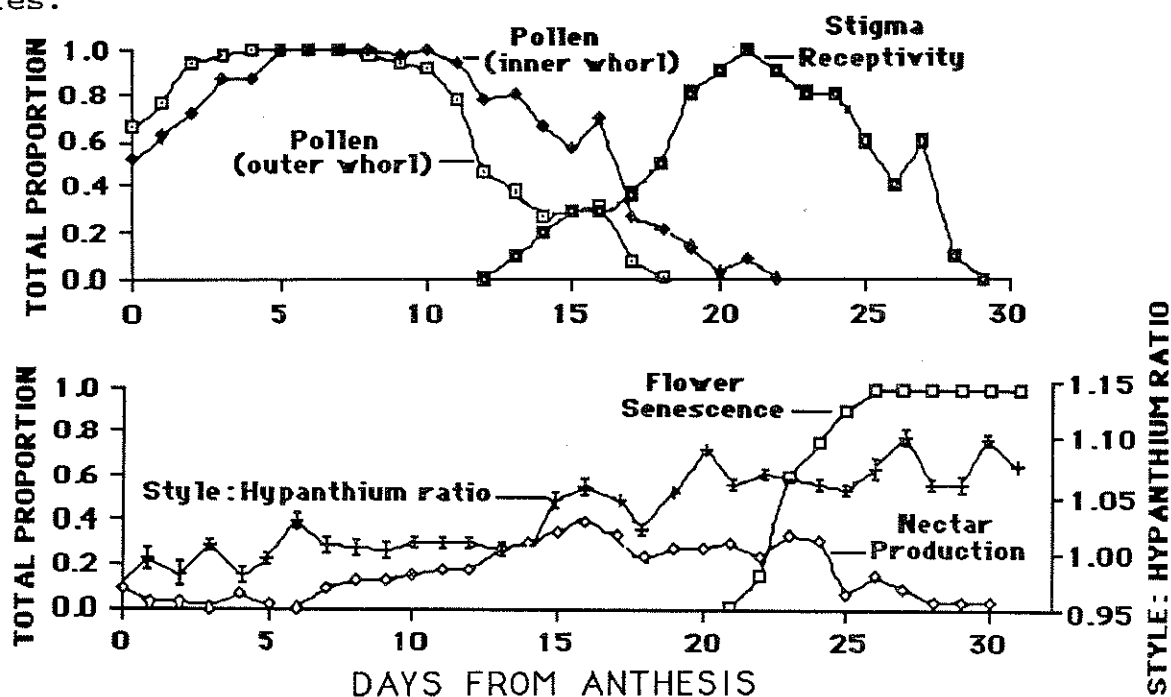


Fig. 2 Post-anthesis floral development of *E. urnigera*.

The proportion of flowers with (top) free pollen on the inner and outer whorls of stamens, a receptive stigma (positive esterase test), (bottom) senescing anthers and the average proportion of the hypanthium filled with nectar and the length of the style relative to the depth of the hypanthium (>1 style extending above hypanthium rim) (Tree 1; n=10).

Within-tree variation in flowering

E. urnigera is at least partially self-fertile (viable self progenies have been grown) and variation in flowering within trees would allow ample opportunity for self-pollination, despite the pronounced protandrous development of individual flowers. The time for buds to begin flowering within individual trees may be spread over approximately 100 days and even within umbels there is considerable asynchrony (up to 60 days) which would further enhance the probability of self-pollination.

Pollinators

E. urnigera is predominantly bird pollinated. Insect activity on the mountain is negligible during winter. For example, the number of strong flying insects (bees, wasps, flies and large moths) trapped near flowers of E. urnigera was only 1.5% of the number trapped over the same period from the lowland, predominantly insect pollinated species, E. morrisbyi. Mammals (e.g. possums Barber 1965), while observed feeding on flowers, do not appear to be important.

The major bird species observed visiting flowering trees at both high and low altitude sites were the New Holland and Crescent Honeyeaters, which together accounted for 98% of all bird visits (n= 1130; over 100 hours of observation) and virtually all observations of flower feeding (Table 1). While the time of observation differed, the New Holland Honeyeaters were more active in the low altitude, green population (70% of flower visits; n= 9907) whereas the Crescent Honeyeaters were more active in the high altitude, glaucous population (57% of flower visits; n=4027).

Foraging pattern

These two species of honeyeaters appear to be the prime pollen vectors in E. urnigera and of all their inter-flower movements, up to 11% were between flowers on different trees. At both high (1100m) and low (800m) altitudes, the mean inter-tree flight distance is marginally greater than the mean distance between

nearest flowering neighbours. Foraging behaviour is thus close to nearest-neighbour, although this appears to be modified by a preference for heavily flowering trees as there is a strong correlation between flower abundance and the number of bird visits. In addition, long-distance (probably escape or exploratory) flights greater than 30m, account for a small (6-9%), but no doubt genetically significant, proportion of inter-tree movements.

Implications for gene flow

Pollinator flight distances were used as an indirect estimate of pollen-mediated gene flow. However, pollinator foraging movements may underestimate gene flow (Levin 1981), particularly if pollen carry-over is important. In the present case, birds visited up to a mean of 16 flowers per tree. Flower probing suggested that the mean maximum pollen carry-over was 47 flowers ($n=10$), although this is clearly an overestimate of effective pollen carry-over and the shape of the deposition curve needs to be determined. Nevertheless, estimates of the standard deviation for pollen-mediated gene flow (s_p) from this study, assuming that the distribution of pollinator flight distances and gene flow distances are concordant, are approximately 18m in both high and low populations. Inter-tree movements greater than 30m were treated as equal to 50m, which is reasonable as the corresponding curves have approximately 9% of distances greater than 30m. These estimates correspond to absolute as opposed to axial variance estimates (Crawford 1985). However, it is the directional, axial variance which is necessary for the estimation of neighbourhood size and gene flow along the cline (Crawford 1985; $\sigma^2_{\text{absolute}} = 2\sigma^2_{\text{axial}}$) and this yields a standard deviation of approximately 13m.

Both our absolute and axial estimates of pollen-mediated gene flow are substantially less than Barbers' (1965) suggestion for the "mean free pollen path" of 100m and roughly comparable with Endlers' suggestion of total gene flow ($\sigma^2_{\text{seed}} + \sigma^2_{\text{pollen}}$) of 20m.

Using estimates of seed-mediated dispersal from a mallee eucalypt (*E. risdonii* $\sigma_s = 4.6\text{m}$; Potts and Reid 1988) and assuming an outcrossing rate of 0.7 (see Moran and Bell 1983), then following van Dijk (1985), rough estimates of the standard deviation (σ) for total gene flow ($\sigma^2 = 1/2 t \sigma_p^2 + \sigma_s^2$) in *E. urnigera* would be of the order of 9 m.

Conclusions

Restricted pollinator movements thus suggest that actual pollen-mediated gene flow along this cline may be far more limited than previously suggested. This would be further accentuated by clinal variation in flowering time. While we have no doubt underestimated actual pollen-mediated gene flow, even if doubled our estimates still suggest relatively restricted pollen-mediated gene flow. This accords with the low percentage of alien glaucousness phenotypes found in open-pollinated seedlings grown from populations on either side of the transition zone (Fig. 1). Clearly, gene flow along clines in eucalypts should be reassessed

Acknowledgements

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